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Use of a newly isolated extreme thermophile for the production of 2nd generation bio-ethanol

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Background and aim

Bio-ethanol produced by microbial fermentation is currently recognized as one of the most promising candidates for replacing fossil fuels. The use of residual biomass as the substrate is recommended, in order to minimize the impact in land use and greenhouse gas emissions. However, lignocellulose can be composed of up to 25 % of pentose sugars, which are typically not degraded by the currently used industrial ethanologenic strains. The goal of this work is to isolate an extreme thermophilic microorganism capable of stoichiometric conversion of pentoses to ethanol, and use it for 2nd generation bio-ethanol production.

Questions

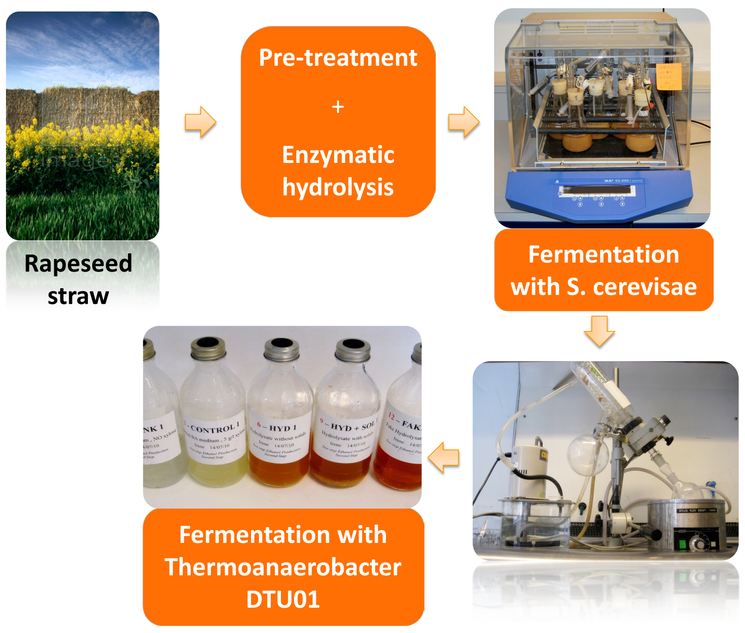
- Can all the fractions of a pre-treated lignocellulosic substrate be used in the process?
- Is the new isolate tolerant to typical inhibitors derived from pre-treatment of lignocellulose?
- Can the process be improved if combined with fermentation by *Saccharomyces cerevisae?*

Characteristics of the new isolate

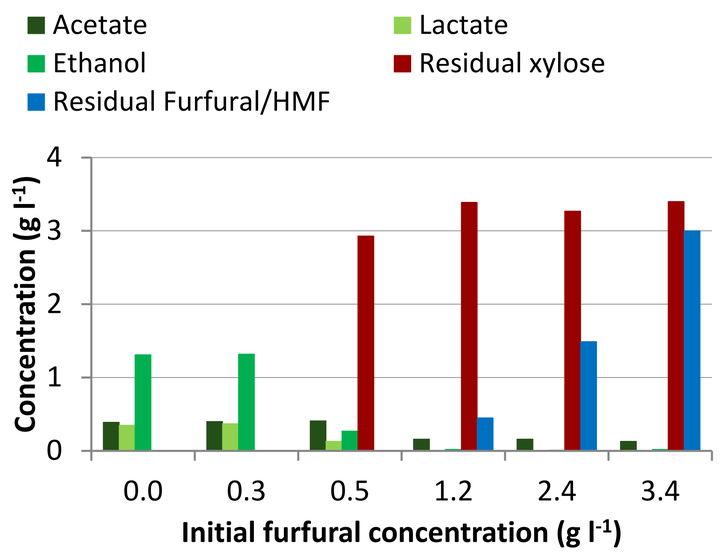
Isolation source	Thermophilic UASB reactor for hydrogen production from household waste
Genus	*Thermoanaerobacter*
Morphology	0.5-2 µm rods, spore-forming
Opt. growth conditions	70° C, pH 7
Substrates utilized	Glucose, xylose, arabinose, sucrose, cellobiose, inulin, starch, xylan, pectin
Ethanol yield	0.43 $g/g_{consumed\ xylose}$
Other products	Acetate, lactate, hydrogen

Methods overview

- Isolation using Hungate roll tubes
- Batch tests using basic anaerobic medium



Inhibitor tests



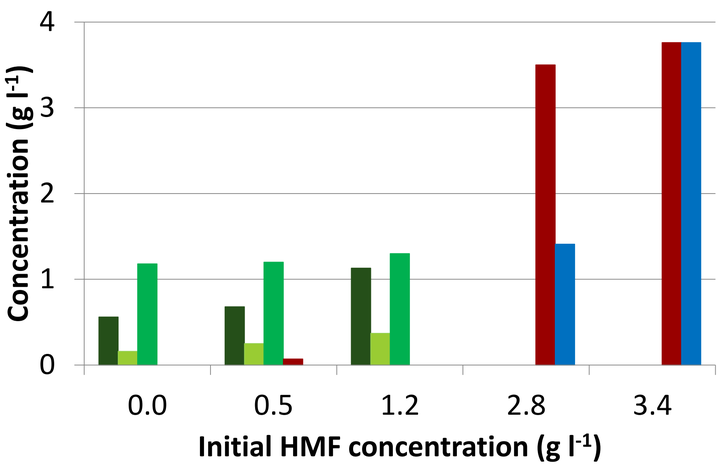


Fig. 1. End product and residual xylose and inhibitor concentrations after *T.* DTU01 fermentation of 5 g l^{-1} xylose in the presence of different concentrations of the inhibitors furfural (A) and hydroxymethyfurfural (HMF) (B).

2-step fermentation

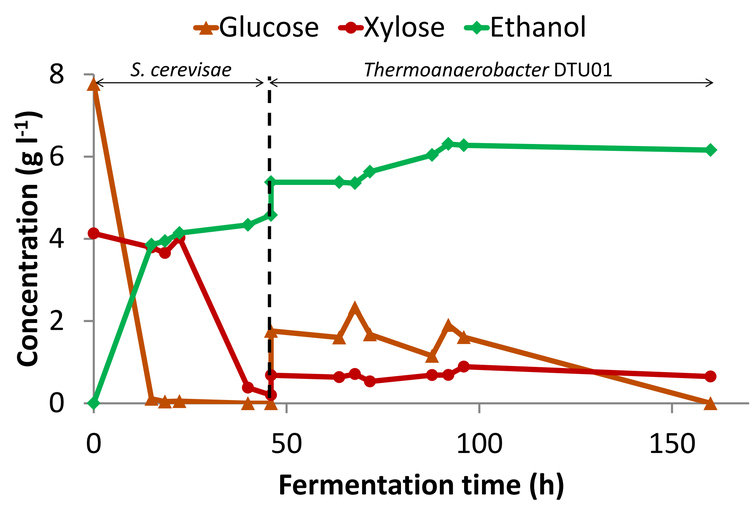


Fig. 2. Glucose, xylose and ethanol profiles during a two-step fermentation process with *S. cerevisae* and *Thermoanaerobacter* DTU01, using pre-treated rapeseed straw (5 % solid loading, as well as the liquid fractions of the pre-treatment) as substrate. The dashed lines refer to a control batch where only strain DTU01 was used (only one fermentation step).

Strains used	Ethanol yield (g/g total sugars)
S. cerevisae	0.38
***T.* DTU01**	0.29
***S. cerevisae* + *T.* DTU01**	0.44

Table 1. Comparison of ethanol yields from pre-treated rapeseed straw in a batch fermentation if using *S. cerevisae* and *Thermoanaerobacter* DTU01 individually or in a sequential 2-step process.

Conclusions

- The new isolate, *Thermoanaerobacter* DTU01, could produce the **highest ethanol yield from xylose reported so far for a wild type strain, 0.43 g g^{-1}**;
- Low amounts of inhibitors seem to be **beneficial** for the strain; furfural is more toxic than hydroxymethylfurfural (HMF);
- *Thermoanaerobacter* DTU01 could produce ethanol from **all the fractions** of pre-treated rapeseed straw;
- A 2-step process with *Thermoanaerobacter* DTU01 and *S. cerevisae* was **more efficient in producing ethanol from rapeseed straw** than the separate counterparts.